

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

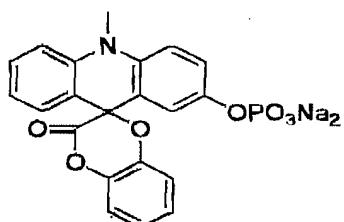
AMENDMENT TO THE CLAIMS

1-7. (Cancelled)

8. (Currently amended) The chemiluminescent substrate of claim 4
43 wherein said counter ions A are selected from the group
 consisting of CH_3SO_4^- , FSO_3^- , CF_3SO_3^- , $\text{C}_4\text{F}_9\text{SO}_3^-$, $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$, halide,
 CF_3COO^- , CH_3COO^- , and NO_3^- .

9-21. (Cancelled)

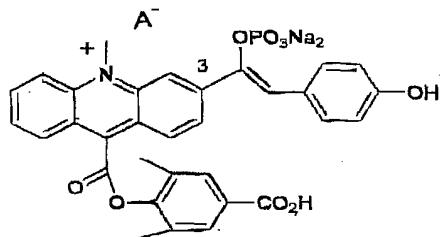
22. (Currently amended) The chemiluminescent substrate of claim
21-61 having the following structure:



23-24. (Cancelled)

25. (Currently amended) The A chemiluminescent substrate of
claim 23 having the following structure,

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651



wherein A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said counter ion A^- is selected from the group consisting of $CH_3SO_4^-$, FSO_3^- , $CF_3SO_3^-$, $C_4F_9SO_3^-$, $CH_3C_6H_4SO_3^-$, halide, CF_3COO^- , CH_3COO^- , and NO_3^- .

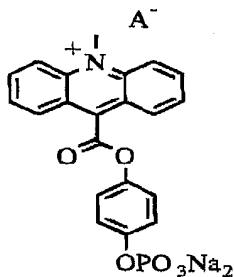
-5-

WEINGARTEN, SCHURGTN,
 GAGNERIN & LEBOVICI LLP
 TEL. (617) 542-2280
 FAX. (617) 451-0313

Application No. 09/626,566
Filed: July 27, 2000
Group Art Unit: 1651

26-28. (Cancelled)

29. (Currently amended) The A chemiluminescent substrate of claim 26 having the following structure:

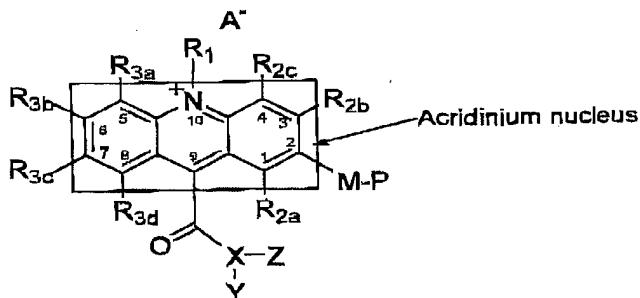


wherein A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said counter ion A⁻ is selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻, and NO₃⁻.

30-42. (Cancelled)

43. (Currently amended) The A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651



wherein

P is PO_3Na_2 or a sugar moiety;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} , are hydrogen;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

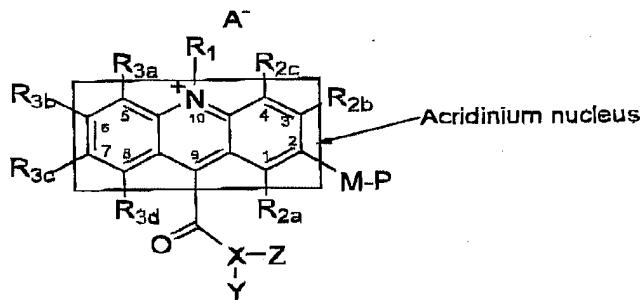
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

44. (Currently amended) The A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure,



wherein

P is PO_3Na_2 or a sugar moiety;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} , are hydrogen;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

Application No. 09/626,566
Filed: July 27, 2000
Group Art Unit: 1651

X is O; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

45. (Previously added) The chemiluminescent substrate of claim 43, wherein

P is PO_3Na_2 ;

X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

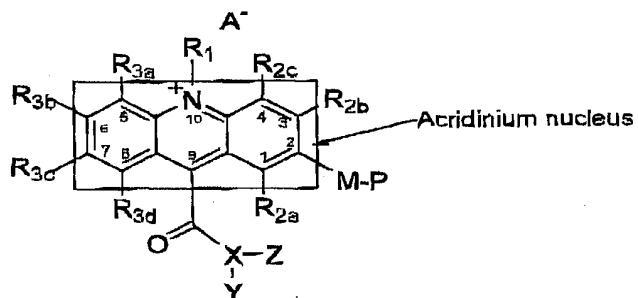
46. (Previously added) The chemiluminescent substrate of claim 43, wherein

P is PO_3Na_2 ;

X is S; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

47. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651



wherein

P is PO_3Na_2 or a sugar moiety;

M is oxygen;

R₁ is selected from the group consisting of sulfoalkyl and carboxymethyl;

R_{2a}, R_{2b}, R_{2c}, R_{3a}, R_{3b}, R_{3c} and R_{3d}, can be the same or different, selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-

-10-

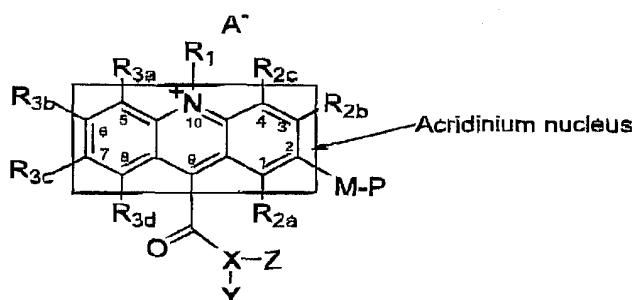
WEINGARTEN, SCHURGIN,
 GAGNEBIN & LEBOVICI, LLP
 TEL. (617) 542-2290
 FAX. (617) 451-0313

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

48. (New) The chemiluminescent substrate of claim 47 wherein said counter ions A⁻ are selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻, and NO₃⁻.

49. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

Application No. 09/626,566
Filed: July 27, 2000
Group Art Unit: 1651

P is selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg and $\text{C}(\text{=O})\text{R}$ group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

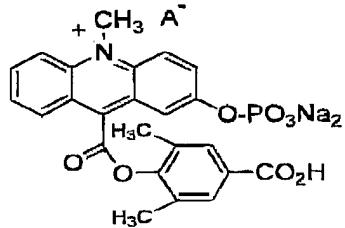
when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl,

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

(2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl,; and Z is omitted; and when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

50. (New) The chemiluminescent substrate of claim 49 wherein said counter ions A⁻ are selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻, and NO₃⁻.

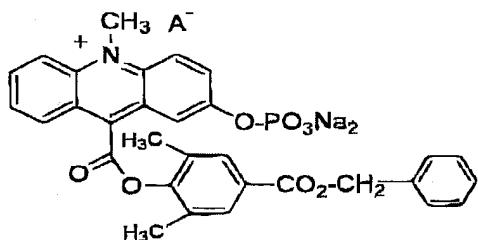
51. (New) The chemiluminescent substrate of Claim 43 having the structure,



wherein A⁻ is selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻, and NO₃⁻.

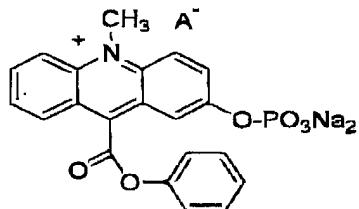
Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

52. (New) The chemiluminescent substrate of Claim 43 having the structure,



wherein A- is selected from the group consisting of CH_3SO_4^- , FSO_3^- , CF_3SO_3^- , $\text{C}_4\text{F}_9\text{SO}_3^-$, $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$, halide, CF_3COO^- , CH_3COO^- , and NO_3^- .

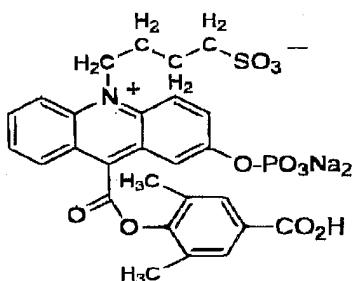
53. (New) The chemiluminescent substrate of Claim 43 having the structure,



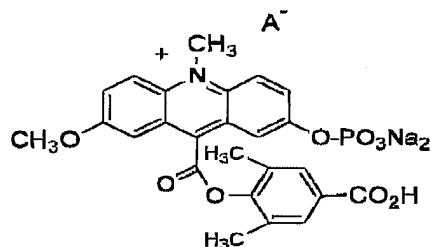
wherein A- is selected from the group consisting of CH_3SO_4^- , FSO_3^- , CF_3SO_3^- , $\text{C}_4\text{F}_9\text{SO}_3^-$, $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$, halide, CF_3COO^- , CH_3COO^- , and NO_3^- .

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

54. (New) The chemiluminescent substrate of Claim 43 having the structure



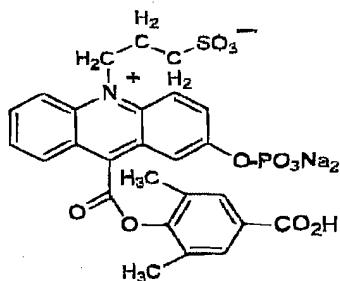
55. (New) The chemiluminescent substrate of Claim 47 having the structure,



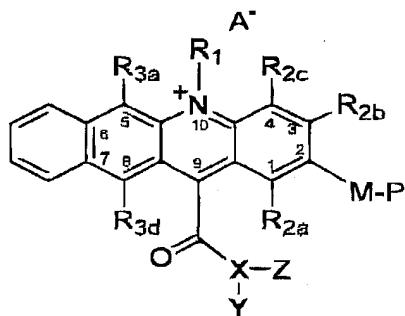
wherein A- is selected from the group consisting of CH_3SO_4^- , FSO_3^- , CF_3SO_3^- , $\text{C}_4\text{F}_9\text{SO}_3^-$, $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$, halide, CF_3COO^- , CH_3COO^- , and NO_3^- .

56. (New) The chemiluminescent substrate of Claim 43 having the structure

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651



57. (New) A chemiluminescent substrate of having the structure



wherein

P is selected from the group consisting of PO_3H_2 ,

PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and $\text{C}(\text{=O})\text{R}$ group

wherein R is an alkyl group having 1 to 6 carbon atoms;

Application No. 09/626,566
Filed: July 27, 2000
Group Art Unit: 1651

M is oxygen;

R₁ is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a}, R_{2b}, R_{2c}, R_{3a}, and R_{3d}, can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

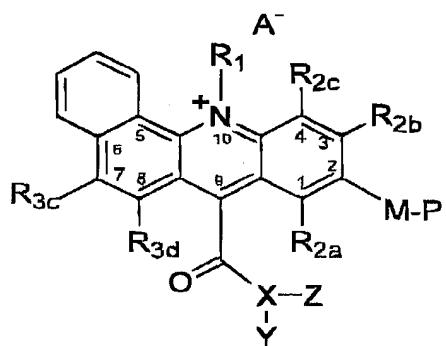
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

58. (New) A chemiluminescent substrate having the structure



wherein

P is selected from the group consisting of PO_3H_2 , PO_3K_2 ,

$\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and $\text{C}(\text{=O})\text{R}$ group

wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3c} and R_{3d} , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN) ;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

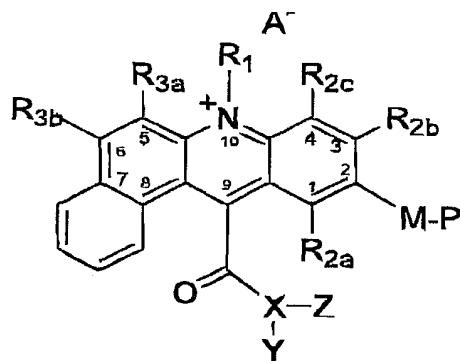
present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

59. (New) A chemiluminescent substrate having the structure



-19-

WEINGARTEN, SCHURGIN,
 GAGNEDIN & LEBOVICI LLP
 TEL. (617) 542-2290
 FAX. (617) 451-0313

Application No. 09/626,566
Filed: July 27, 2000
Group Art Unit: 1651

wherein

P is selected from the group consisting of PO_3H_2 , PO_3K_2 ,

$\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and $\text{C}(=\text{O})\text{R}$ group

wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , and R_{3b} can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

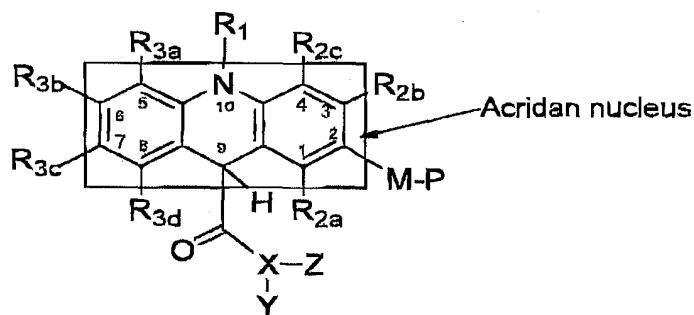
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl,; and Z is omitted; and
 when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

60. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and $\text{C}(\text{=O})\text{R}$ group
 wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

Application No. 09/626,566
Filed: July 27, 2000
Group Art Unit: 1651

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

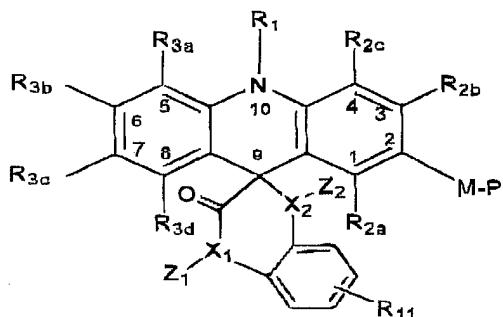
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651

61. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and $\text{C}(\text{=O})\text{R}$ group
 wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group

Application No. 09/626,566
Filed: July 27, 2000
Group Art Unit: 1651

that can form an anion and pair with the quaternary ammonium cationic moiety; and

X_1 and X_2 are the same or different and are selected from the group consisting of O, N or S, such that,

when X_1 and X_2 are O or S, R_{11} is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl, halides, nitro, sulfonate, sulfate, phosphonate, $-CO_2H$, $-C(O)OR$, cyano (-CN), -SCN, -OR, -SR, -SSR, $-C(O)R$, $-C(O)NHR$, ethylene glycol, or polyethylene glycol, where R is as defined above; and Z_1 and Z_2 are omitted; and

when at least one of X_1 and X_2 is N, Z_1 and Z_2 are toluenesulfonyl, and R_{11} is carboxypropyl.